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Study of Analysis of Induced Biochemical Changes in Infected *Calotropis gigantea* Plants

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ABSTRACT
Plants of Calotropis gigantea infected with chlorotic leaf spots mosaic like symptoms were collected and the infection was maintained on different host plants. These plants were evaluated for biochemical changes due to viral infection and were screened for different phytochemical parameters. Significant variations among infected and healthy plants were observed i.e. total chlorophylls (1.70-1.78 mg/g), chlorophyll b (0.43-0.49 mg/g), carotenoids (0.96-0.98 µg/g), total phenols (0.62-0.68 mg/g), simple phenols (0.61-0.66 mg/g) and ascorbic acid (4.25-5.17 mg/100g). Chlorophyll-a, b, total Chlorophylls and carotenoids were observed to be decreased in the infected leaves as compared to healthy leaves, while total phenols, simple phenols and ascorbic acid increased in infected leaves as compared to healthy leaves.
Keywords: *Calotropis gigantea*, virus, infected, chlorotic, mosaic.

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INTRODUCTION

Herbal plants are an important part of our natural wealth. They are being used from very ancient times till the present day. The remarkable therapeutic diversity of herbal plants is one of the main reasons of their distinct position. *Calotropis gigantea* is also a plant of herbal importance. This plant *Calotropis gigantea* (Botanical name) is known by different names in English Crown flower, giant Indian milkweed, in *Hindi* Aak*, Arka*, *Madar,* in *Sanskrit Ganarupa, Mandara, Vasuka, Svetapushp etc.* In India it has other names *Ekka* (Kannada)*, Erukku* (Tamil and Malayalam) and *Jilledi Puvvu* (Telugu)*.* It is also known by different vernacular names like, French cotton, Alarka, Rooster tree, Widuri in different parts of world. It is commonly called as *Arka* in most of the regions of India. The genus *calotropis* R.Br. (Asclepiadaceous) is distributed in tropical and subtropical regions of Asia and Africa [1].

This plant belongs to Apocynaceae family which includes latex bearing plants. It is a common wasteland weed commonly found in India. *Arka* is drought resistant, salt tolerant to a relatively high degree, grows wild to an altitude of 900 meters throughout the country [2] with annual rainfall of 300-400 mm. It is used as an ancient medicinal plant for curing many diseases. In India, there has been interest in the potential of medicinal plant for development of drugs having wound healing properties as taught in a popular form of Indian medicine known as Ayurveda [3]. *Arka* is a common medicinal plant in Indian subcontinent having purgative subcontinent, alexipharmic, anthelmintic, analgesic, anticonvulsant, anxiolytic, sedative and antipyretic effect (4, 5) and is used as a treatment for leprosy, leucoderma, ulcers, tumours, piles and diseases of the spleen, liver and abdomen (6).

Arka has latex which contains the cardiac glycosides, calotopin, uscharin, calotoxin, calactin, uscharidin and gigantin. The latex is also used as caustic, acrid; expectorant, depilatory, anthelmintic; useful in leprosy scabies, ring worm of the scalp, piles, eruptions on the body, asthma, enlargement of spleen and liver, dropsy; applied to painful joint swellings (7). So this plant is useful for many purposes.

Calotropis gigantea is known for its utilization in traditional medicinal system for various properties to cure a variety of diseases. Herbal medicines have less side effects and man can get the herbs easily from nature. India being a tropical country is blessed with vast natural resources and ancient knowledge for its judicious utilization (8). In India two species viz. *C. procera* and *C. gigantea* are available. These two species show similar botanical aspects and pharmacological effects. In Ayurvedic medicine the plant *C. gigantea* is known as "*Sweta Arka"* and *C. procera* as "*Raktha Arka."* The only difference between these is

in the colour of the flowers; however, they are white in *C. procera* and pinkish white in *C. gigantea*. The plant is an erect, tall, large, much branched perennial shrub or small tree. It generally grows to a height of 4 meters.

Leaves of this plant have been observed with yellow chlorotic and some mosaic like symptoms. Multiplication of virus particles in the infected plant cells alters biochemical compounds of cells such as chlorophyll, carotene, organic carbon, nitrogen, protein, phosphorous proteins, phenolic compounds and nucleic acids etc. (9). External manifestations of disease symptoms are the results of altered host metabolism. The extent of crop loss is mainly associated with severity of visible symptoms (10). The present study was undertaken to determine changes in phytochemical constituents *viz.* chlorophylls, carotenoids, phenols and ascorbic acid in *C. gigantea* due to viral infection as compared to healthy plants. The study is also carried out to determine antioxidant properties in healthy as compared to infected plants. The molecular agents that prevent the oxidation of other molecules either by stopping the transfer of electrons or hydrogen are known as the antioxidants. Antioxidants can protect the human body from free radicals and Reactive Oxygen Species (ROS) effects. Oxidative damages caused by free radicals to living cells mediate the pathogenesis of many chronic diseases such as Parkinson's, Alzheimer's, cancers, aging, cardiovascular, atherosclerosis, cataract, inflammatory, and other degenerative ailments (11). Phenols, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts, the antioxidant activities of which play an important role in the absorption or neutralization of free radicals (12).

The presence of many phytochemicals such as Usharin, gigantin, calcium oxalate, α and β-calotropeol, beta-amyrin, fatty acids (both saturated and unsaturated), hydrocarbons, acetates and the benzoates, a mixture of tetracyclic triterpene compounds and giganteol whereas flavonoids, triterpenoids, alkaloids, steroids, saponins, terpenes, esters of calotropeols, volatile long chain fatty acids, glycosides and proteases have been isolated in different parts of *C. gigantea* especially in the leaves (13).

In India *calotropis* occupies special importance because of its large industrial uses and economic values. It has various medicinal properties. Different parts of the plant have immense potential to cure various diseases and disorders like asthma, cold, epilepsy, fever, indigestion, leprosy, piles, skin diseases etc., and exhibiting activities that are anti-inflammatory, anthelmintic, anticancer and antitumor; as observed in various polyherbal preparations (14). It is a highly potential plant resource and different parts of this plant are used for multi purposes. The various uses of this plant are biogas production, substitute for petroleum products, cleansing of water, energy plantation, fibers, fodder, latex or rubber, substitute for paper etc. So, in order to understand their pharmacological action, there is a need to scientifically evaluate them at molecular and biochemical level.

In nature plants are often exposed to different types of environmental stresses. Crops can be affected by these stresses resulting in the reduction in the yields. Virus infections can drastically reduce crop yield (15), resulting in economic losses. Abiotic stresses include the various environmental conditions like temperature, heat and chemical stresses. Biotic stresses include infection by pathogens (including bacteria, fungi, viruses and nematodes) and attack by herbivore pests (16). Wild plants are almost colonized by a number of microbes, including fungi, bacteria and viruses and which may cause any of these interactions (17). Viral diseases in perennial crop plants are more dangerous than in annual crops (18). Pathogens affect host populations by reducing viability, fecundity and competitive ability, as well as affecting community interactions (19). In crops, virus infection can reduce plant growth by depressing photosynthesis and changing metabolism (20). In case of *calotropis*, infection has been reported due to bacterial, fungal and viral pathogens. Infected plants show systemic brilliant chlorotic to yellow spots on the leaves suspected to be due to virus infection based on the nature of symptoms.

This plant is seen susceptible to infection by several viruses viz. *Groundnut bud necrosis virus* (GBNV) (21) and a *Begomovirus* (22). Plants in the field were observed to exhibit systemic yellow spots symptoms. In infected plants the flowers setting and size was reduced and fruits became discolored, shriveled and sterile which died prematurely whereas the seed was four times smaller that fails to germinate. The overall plant height and vigour were markedly reduced. Undulated margins and spots that coalesce into yellow blotches along the veins in infected leaves have been observed. Furthermore leaves become thin and puckered giving the plant a distorted appearance. Keeping in view the medicinal importance of *Arka*, there is a need to work towards understanding epidemiology of the disease as well as effects on phytochemical constituents. Phytochemical constituents are important to understand their roles in the plants and to devise strategies for rapid diagnosis and control. Further, the effect of virus infection on active phytochemical constituent concentration is important to understand the importance of this disease.

MATERIALS AND METHODS

Collection of infected *Calotropis gigantea* samples

Plants of *Calotropis gigantea* infected with chlorotic leaf spots mosaic like symptoms were collected and maintained at glass house under suitable conditions at ± 20°C. The *Calotropis gigantea* leaves that showed the symptoms were used in this study as the source of inoculum. The infection was maintained on different host plants.

Inoculation of test plants

For inoculation ice cold 100 mM potassium phosphate buffer (pH 7.4) was sprinkled on the test or host plants. Then carborundum was dusted on these plants and after that potassium phosphate buffer was spread on them. The infected leaves of *C. gigantea* were transferred in a pestle mortar and crushed in the same buffer. This crushed suspension of infected plant was applied on the surface of leaves of host plants. Finally distilled water was sprinkled on the plants to remove the secondary metabolites from the surface.

BIOCHEMICAL ANALYSIS

Chlorophyll, carotenoids and ascorbic acid content were determined in fresh leaves of *C*. *gigantean*. However, total phenols (simple phenols and tannins) were analyzed in oven dried leaves (green leaves dried at 45°C for 3 hours). Chlorophyll and carotenoids were estimated by the method of Jayraman (23) and Davies (24). Ascorbic acid in fresh leaves was estimated by AOAC (25). Total phenols were estimated by the method of Makkar (26).

Chlorophyll and carotenoids estimation

For total chlorophyll, extraction was carried out into cleaned pestle and mortar by grinding sample of *Calotropis* leaves (0.2 g) with 80 per cent acetone. The extracts were centrifuged at 4,000 rpm for 1 minute and content was re-extracted 4 times in the same manner with 80 per cent acetone until residues became colorless. Finally, this volume was made up to 20 ml with 80 per cent acetone and absorbance was measured at 663 nm, 645 nm and 480 nm on spectrophotometer model Merck Spectroquant Pharo 100.

Preparation of standard for estimation of ascorbic acid

Accurately 100 mg of L-ascorbic acid was dissolved in 500 ml of 1.0 per cent oxalic acid solution which was used as standard (always prepared fresh). The amount of ascorbic acid in sample is expressed as mg/100g.

Estimation of ascorbic acid

Fresh, just after plucking *Calotropis* leaves samples (25 g) was accurately weighed, and ground with 25 ml of 2.0 per cent oxalic acid as extraction medium in order to get slurry. The total weight of slurry was recorded. 10 g slurry was taken in a 100 ml beaker and volume was made up to 25 ml with 1 per cent oxalic acid. This content of the beaker was filtered properly through Whatman Filter paper No.1 and 5.0 ml of this filtrate was titrated against 2,6 dichlorophenol indophenol dye. From these titrations three concordant readings were observed.

Estimation of phenolic compounds

Extraction: Finely ground 0.2 g sample was taken in a 100 ml beaker. To this sample, added 10 ml of 70 per cent acetone and kept it on a shaking water bath for 2 hrs at 30°C. The beaker was tightly covered to avoid evaporation. After the expiry of time contents were centrifuged at 10,000 rpm for 20 minutes and supernatant was used for the estimation of total phenols.

Estimation: 0.1 ml aliquot was taken and final volume was made upto 1ml with distilled water. To this 2.5 ml of 20 per cent Na_2CO_3 followed by 0.5 ml of Folin Ciocalteu reagent (1N) was added. Content was incubated for 40 minutes at room temperature. Developed Blue colour was recorded at 725 nm on spectrophotometer model Merck Spectroquant Pharo 100.

Antioxidant Activity

DPPH radical scavenging activity

DPPH radical scavenging activity or 2,2-diphenyl-1- picrylhydrazyl free radical scavenging activity was evaluated by the methods of Kordali et al. (27) and Sharma and Bhatt (28). It was calculated in the aqueous extracts of samples and for standard, monomer of (+) catechin was used.

The absorbance was recorded at 517 nm with spectrophotometer model Merck Spectroquant Pharo 100 after 30 minutes incubation in the dark at 30°C. The percentage of DPPH free radical scavenging activity (% inhibition) was calculated with the help of following equation:

$$\% \text{ inhibition} = \frac{Abs\ (Control) - Abs\ (Test)}{Abs\ (Control)} \times 100$$

IC_{50} value (the amount of antioxidant necessary to decrease the initial DPPH free radical concentration by 50 per cent) was calculated from the regression line obtained from the plot of per cent inhibition against concentration of each solution using the following equation:

$$IC_{50} \text{ value} = \frac{(50 - \text{y intercept})}{\text{Slope}}$$

Extraction of total flavonoids from the sample

Flavonoids were extracted by following method of Swain and Hills (29); Mahadevan and Sridhar (30). The dried leaf sample (1.0 g) was taken in a test tube and 10 ml of methanol was added to it. The test tubes were placed on a water bath shaker maintained at 37°C for shaking. After 12 hours, the test tubes were removed and centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected and evaporated to dryness. The resulting extract was re-dissolved in 1ml of methanol for further use.

Estimation of total flavonoids

An aliquot of 250 µl of redissolved methanol extract was used for the estimation of flavonoids. The intensity of pink colour developed was measured at 510 nm and the result was expressed as mg of (+) Catechin equivalent (CEs) per g of extract using following formula:

$$\text{Flavonoids (mg/g)} = \frac{\text{Absorbance at 510} - \text{y intercept}}{\text{Slope}}$$

Y intercept and slope were calculated from the standard curve drawn with (+) Catechin.

Estimation of quinones

Quinones were estimated by using fresh leaf samples of *C. gigantea* by using catechol as a standard by the method of Mahadevan (31) followed by Mahadevan and Sridhar (30).

Estimation of Polyphenol Oxidase (PPO) activity

Polyphenol oxidase activity of fresh *C. gigantea* leaves was estimated in freshly prepared acetone powder by the method of Farkas and Kirlay (9).

Preparation of acetone powder

Accurately weighed 4.0 g fresh *C. gigantea* leaves were transferred to a cleaned pre-chilled pestle and mortar. Then chilled acetone (kept at -20°C) was added just enough to cover the sample, which was ground slowly in clockwise direction for 2-3 minutes, followed by fast anticlockwise grinding for 3-5 minutes. The contents were filtered through Whatman No.1 filter paper and washed repeatedly with chilled acetone till a colourless filterate was obtained. The residue was air dried on the filter paper and immediately stored in air tight sealed vials at - 4°C for further use.

Preparation of Enzyme extract

To a clean centrifuge tube kept in ice cold conditions, 0.1 g of acetone powder was added, followed by 4.0 ml of double distilled water. The content was vortexed and centrifuged in cold at 4,000 rpm for 10 minutes (4°C). The supernatant containing water-soluble enzyme was filtered through cotton and collected into a test tube, already placed in icebox. The residual bound enzyme in the pellet were re-extracted with 5.0 ml of 0.2 M $NaSO_4$ solution and centrifuged at 4,000 rpm for 10 minutes at 4°C. The resulting supernatant was collected similarly and pooled with the previous lot. The contents were mixed properly to get the enzyme extract for further use. This was termed as enzyme extract.

Enzyme assay with Pyrogallol as a substrate

Two ml of enzyme extract was taken in a test tube (already placed in icebox) to which 2.0 ml of 0.2 M phosphate buffer (pH 6.0) and 0.5 ml of 1.0 per cent pyrogallol solution (E. Merck, India) as substrate was added. Enzymatic activity (OD/minutes) was observed at 410 nm at 30 seconds interval for 3 minutes on spectrophotometer model Merck Spectroquant Pharo 100.

RESULTS AND DISCUSSION

Chlorophylls are green photosynthetic pigments in all photosynthetic autotrophic organisms and provide characteristic colour to leaves. It is used in treating diabetic foot ulcer, constipation and also used to neutralize the acidifying and stimulating effect of excess protein, sugars and starch. Adding chlorophyll rich food to diet fortifies human body against health disorders (32). Magnesium in chlorophyll improves immune potential by increasing phagocyte activity. The prevention of indirect and direct carcinogenesis by chlorophylls is attributed to their ability to act as powerful antioxidants (33). The total chlorophyll content in the different host plants ranged from 1.70-1.78 mg/g. The maximum value of total chlorophyll was observed in healthy *C. gigantea* leaves (control) while different host species had lower value than the healthy. All the host range species were statistically significant with each other.

Chlorophyll 'a' is 'real life force' of living beings, besides synthesizing food. The chlorophyll 'a' content was statistically non significant in all of the host range species. It is a great source of vitamins, minerals and other phytochemicals. Chlorophyll is made up of two classical components, chlorophyll 'a' (bluish black) and chlorophyll 'b' (dark green). The chlorophyll 'a' was non significant while chlorophyll 'b' content

ranged from 0.43-0.49 mg/g, respectively. The maximum value of chlorophyll 'a' and 'b' was observed in healthy *Calotropis* (control) leaves while other had less values as compared to healthy. In this case, all the species were found statistically significant.

Carotenoids are hydrocarbons belonging to the class of tetraterpenoids (C_{40}). They are accessory pigments in photosynthetic system and give characteristic colour to plant parts particularly flowers and fruits. Total carotenoids are widely recognized as nutritional component essentially needed for metabolisms regulating processes in human beings (34). Carotenoids are being considered as potential cancer preventing agent (10) and higher intake of β-carotene may reduce risk of cancer (35). Carotene associated with chlorophyll is converted into vitamin A, which is essential for normal growth, eye-sight and healthy skin. The total carotenoids were ranged in 0.96-0.98 µg/g. The healthy *Calotropis* leaves were observed higher value of carotenoids as compared to other species. All the species were statistically significant with each other.

Ascorbic acid is vitamin C (L-ascorbic acid), fulfils essential metabolic functions in the life of animals and plants. It is found in plants, animals and single cell organisms (36). Ascorbate in plants has beneficial influences on various aspects in plants. Through modifying gene expression, ascorbate not only acts to regulate defense and survival but also act via phytohormones to modulate plant growth (37). Improvement of ascorbate content in plants will increase plant stress tolerance, while decreasing ascorbate content will result in stress sensitivity of plants (38). Plants vary considerably in their physiological response to various kinds of environmental stress. To prevent damage caused by pathogenic attack and to acclimatize change in their environment, plants have evolved direct and indirect mechanism for sensing and responding to pathogenic stimuli. Ascorbic acid is found in all eukaryotes including animals and plants and lack completely in prokaryotes except cyanobacteria. Ascorbic acid has antioxidant and cellular reductant properties and plays multifunctional roles in plant growth, development, and regulation of remarkable spectrum of plant cellular mechanisms against environmental stresses. Ascorbic acid plays an important role in resistance to pathogenesis. Multiplication of virus particles in the infected plant cells alters biochemical compounds of cells such as chlorophyll, carotene, organic carbon, nitrogen, protein, phosphorous proteins, phenolic compounds, nucleic acids etc. (Fraser 1987).

Ascorbic acid ranged from 4.25-5.17 mg/100g. The highest value was observed in *Datura stramonium*, one of the hosts and the lower value was observed in healthy *C. gigante*a. All the species except control were statistically at par with each other.

Table:1. Phytochemical components in healthy and infected leaves of *C. gigantea* (values on fresh weight basis)

C. gigantea	Total chlorophyll (mg/g)	Chlorophyll 'a' (mg/g)	Chlorophyll 'b' (mg/g)	Carotenoids (µg/g)	Ascorbic acid (mg/100g)
Infected	1.70 ±0.03	1.28 ± 0.02	0.43 ±0.03	0.96 ±0.02	5.17 ±0.04
Healthy	1.78 ±0.04	1.29 ±0.01	0.49 ±0.02	0.98 ±0.03	4.25 ±0.02

*Values are mean of five determinations with standard deviation **(**±)

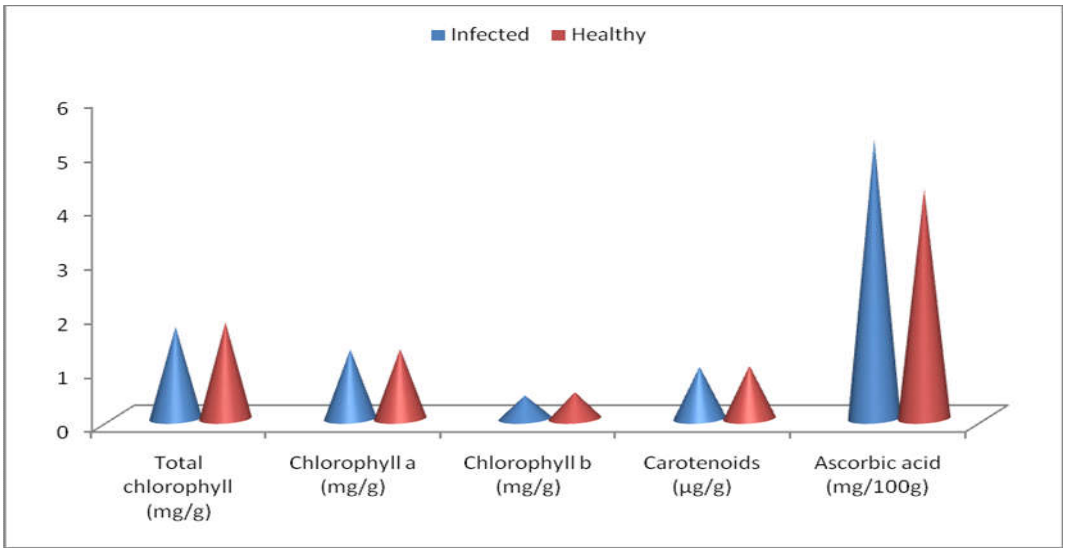

Fig.1. Phytochemical components in healthy and infected leaves of *C. gigantea*

Phenolic compounds are plant's secondary metabolites that constitute one of the most common and widespread groups of substances in them. As stated by Harborne [3], the term "phenolic" or "polyphenol" can be precisely defined chemically as a substance which possesses an aromatic ring bearing one (phenol) or more (polyphenol) hydroxyl substituent, including functional derivatives (esters, methyl ethers, glycosides, etc. (38). Phenolic compounds are one of the major groups of secondary plant metabolites and they are chemically diverse group of substances having diverse functions in plants such as a powerful defense mechanism against different kinds of predators. They also serve as seed germinating inhibitors. Infection of plants induces changes in phenolic substances. Phenols differ in their response and changes, initially an increase in the susceptible and resistant varieties but with the symptom development, phenols decrease in the susceptible varieties whereas phenols accumulate in the resistant varieties. It has been suggested that the major difference between the resistant and susceptible varieties is in the velocity of accumulation of phenols which will be faster in resistance varieties.

Total phenols varied from 0.62-0.68 mg/g. Among all the host plants only *N. rustica* had highest value whereas; the lowest value was recorded in *C. gigantea* (control). Simple phenols were observed from 0.61-0.66 mg/g. The highest value was noticed in infected *Calotropis* plants while lowest value was observed in control i.e. healthy plants.

Antioxidants are substances with free radical chain reaction breaking properties. Among the numerous antioxidants available, flavonoids are naturally occurring phenolic compounds in plants. The antioxidative effect of flavonoids had long been recognized. They are known to inhibit lipid peroxidation to scavenge free radicals and active oxygen, to chelate iron ions and to inactivate lipoxygenase (39).

A significant difference was observed in flavonoid content (Table 2). Values in respect of this parameter showed variation in the range from 18.57 mg/g to 30.39 mg/g. The infected *calotropis* leaves had higher value and lower values were observed in healthy *calotropis* leaves.

Table:2. Phenolic compounds and antioxidant activities in healthy and infected leaves of *C. gigantea* (values on dry weight basis)

C. gigantea	Total Phenols (mg/g)	Simple phenols (mg/g)	Flavonoids (mg/g)	Tannins (µg/100g)	Quinones (mM/min/g tissue)	Antioxidant activity (µg/ml)
Infected	0.67 ± 0.02	0.65 ± 0.03	30.39 ± 0.03	20 ± 0.02	1.71 ± 0.04	24.02 ± 0.04
Healthy	0.63 ± 0.03	0.61 ± 0.04	18.57 ± 0.02	20 ± 0.03	1.56 ± 0.02	1.27 ± 0.03

*Values are mean of five determinations with standard deviation **(**±)

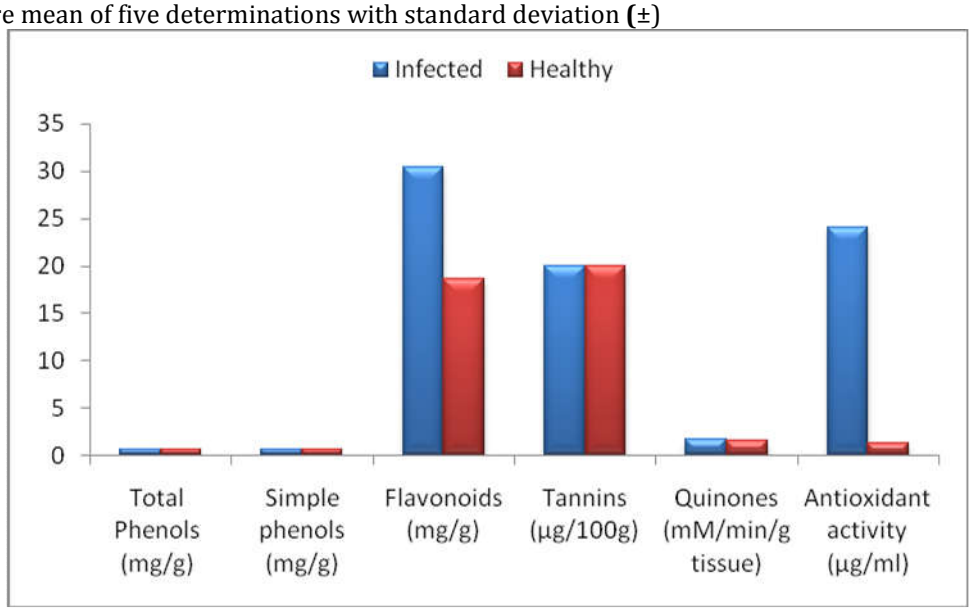

Fig. 2. Phenolic compounds and antioxidant activities in healthy and infected leaves of *C. gigantea*

Antioxidants are the compounds that play an important role in preventing or delaying the onset of major degenerative diseases. Physiologically, these compounds scavenge the free radicals. Free radicals are highly unstable and reactive species which are capable of damaging molecules such as DNA, proteins and carbohydrates. The body is under constant attack from these free radicals formed as a consequence of the body's normal metabolic activities (40). To combat with these radicals, cells are also equipped with an

impressive repertoire of endogenous as well as exogenous (mostly derived from fruits and vegetables) antioxidant molecules.

The status of antioxidant activity in healthy and infected *calotropis* leaves was evaluated and pertinent data are presented in Table 2. Antioxidant activities varied from 1.27 µg/ml to 24.02 µg/ml. Infected leaves showed higher value of antioxidant activity (24.02 µg/ml) while in healthy leaves it was comparatively less (1.27 µg/ml).

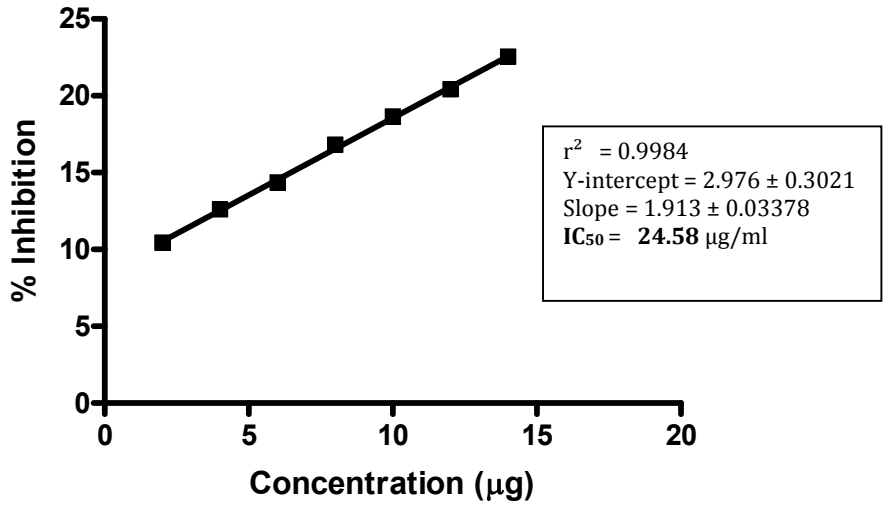

Fig.3. Free radical scavenging activity in infected leaf sample of *C. gigantea*

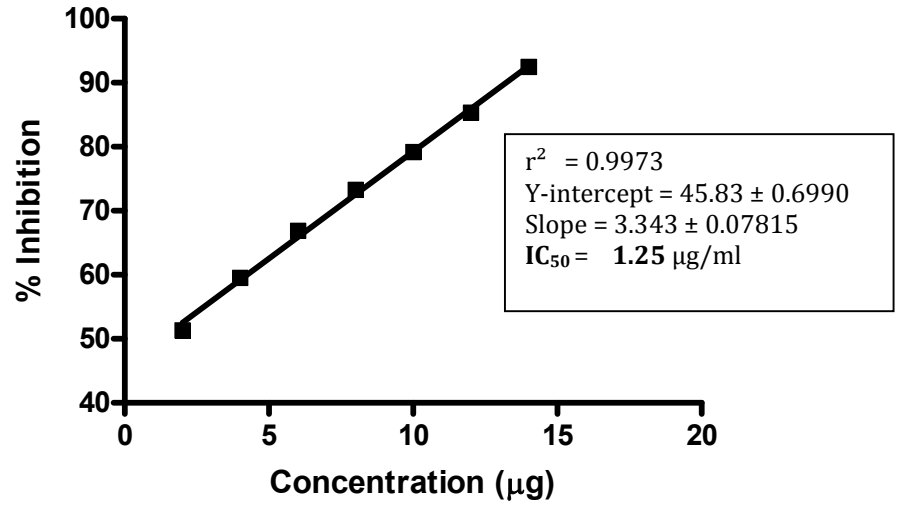

Fig.4. Free radical scavenging activity in healthy leaf sample of *C. gigantea*

Polyphenol oxidase (EC 1.14.18.1), is a copper containing metalloenzyme ubiquitously present in plant kingdom. PPO catalyses the oxidation of variety of phenolic compounds to corresponding quinones which are highly reactive. Polyphenol oxidase (PPO) is a tetramer that contains four atoms of copper per molecule and binding sites for two aromatic compounds and oxygen. It is widely distributed enzyme involved in the biosynthesis of melanins in animals and in the browning of plants. The enzyme catalyzes the oxidation of phenolic compounds to form corresponding quinone intermediates which polymerize to form undesirable pigment (Arnnok et al. 2010).

The PPO activities of fresh leaf samples were measured at interval of 30 seconds up to 3.0 minutes in infected as well as healthy samples as shown in the figure 5. The significant increasing (OD) trend was observed from 0 seconds to 150 seconds after which the enzyme activities decreased in both samples.

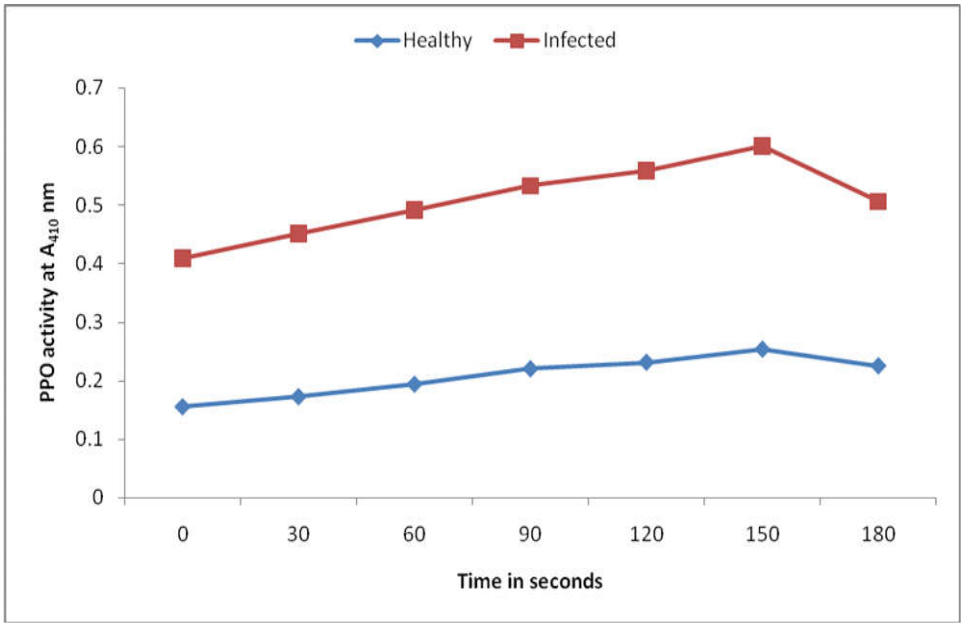

Fig.5. Variation in PPO activity with pyrogallol in leaf sample of *C. gigantea*

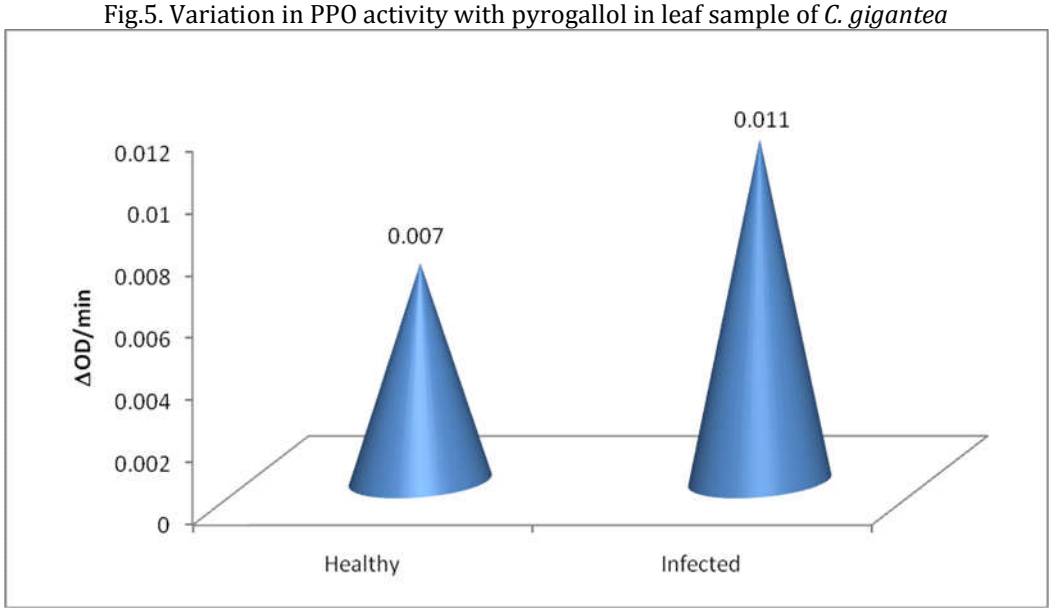

Fig.6**.** Variation in PPO activity (ΔOD/ min) of *C. gigantea* samples

However, higher PPO activity (ΔOD/min) was measured in infected sample while lower PPO activity (ΔOD/min) was observed in healthy *calotropis* leaf sample. PPO (ΔOD/min) activity among the healthy and infected sample is presented in figure 6.

CONCLUSION

In general an increasing trend was observed in infected leaves of *C. gigantea* for phytochemical constituents i.e. antioxidant activity, quinones, flavonoids content, total phenols and ascorbic acid. However, reverse trends over healthy plant i.e. decrease in total chlorophyll and total carotenoids were observed in infected leaves of *C. gigantea*. The PPO showed maximum activity (ΔOD/min) at 150 seconds and higher PPO activity was measured in infected sample in comparison to healthy *calotropis* leaf sample. Phytochemical constituents *viz.* antioxidant activity, quinones, flavonoids, total phenols, ascorbic acid, PPO activity have higher values in infected leaves whereas total chlorophyll and carotenoids had lower values in infected leaves. This could be due to plant's defense mechanism in response to stress or infection.

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